Datasheet for ABIN411361

TNF alpha ELISA Kit

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Overview

Quantity:	96 tests
Target:	TNF alpha
Binding Specificity:	AA 77-233
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	15.6 pg/mL - 1000 pg/mL
Minimum Detection Limit:	15.6 pg/mL
Application:	ELISA

Product Details

Purpose:	Sandwich High Sensitivity ELISA kit for Quantitative Detection of Human TNF alpha
Brand:	PicoKine™
Sample Type:	Cell Culture Supernatant, Serum, Plasma (heparin), Plasma (EDTA), Plasma (citrate)
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Immunogen:	Expression system for standard: E.coli Immunogen sequence: V77-L233
Specificity:	Expression system for standard: E.coli,V77-L233
Cross-Reactivity (Details):	There is no detectable cross-reactivity with other relevant proteins.
Sensitivity:	<1pg/mL

Product Details

Material not included:	Microplate reader in standard size. Automated plate washer. Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection. Clean tubes and Eppendorf tubes. Washing buffer (neutral PBS or TBS). Preparation of 0.01M TBS: Add 1.2g Tris, 8.5g Nacl

Target Details

Target:	TNF alpha
Alternative Name:	TNF (TNF alpha Products)
Background:	Protein Function: Cytokine that binds to TNFRSF1A/TNFR1 and TNFRSF1B/TNFBR. It is mainly secreted by macrophages and can induce cell death of certain tumor cell lines. It is potent pyrogen causing fever by direct action or by stimulation of interleukin-1 secretion and is implicated in the induction of cachexia, Under certain conditions it can stimulate cell proliferation and induce cell differentiation. Impairs regulatory T-cells (Treg) function in individuals with rheumatoid arthritis via FOXP3 dephosphorylation. Upregulates the expression of protein phosphatase 1 (PP1), which dephosphorylates the key 'Ser-418' residue of FOXP3, thereby inactivating FOXP3 and rendering Treg cells functionally defective (PubMed:23396208). Key mediator of cell death in the anticancer action of BCG-stimulated neutrophils in combination with DIABLO/SMAC mimetic in the RT4v6 bladder cancer cell line (PubMed:22517918). . Background: Tumor necrosis factor-alpha(TNF-alpha, or TNF) is secreted by macrophages in response to inflammation, infection and cancer. Human Tumor Necrosis Factor(TNF) and Lymphotoxin(TNF-beta) are cytotoxic proteins which have similar biological activities and share 30 % amino acid homology. TNF-alpha is produced by monocytes, which can stimulate endothelial cells to produce the multilineage growth factor granulocyte-macrophage colony-stimulating factor and extend the role of this immunoregulatory protein to the regulation of hematopoiesis in vitro. TNF is a soluble protein that causes damage to tumor cells but has no effect on normal cells. Human TNF has been purified to apparent homogeneity as a 17.3-kilodalton protein from HL-60 leukemia cells and has showed cytotoxic and cytostatic activities against various human tumor cell lines. The human TNF cDNA is 1585 base pairs in length and encodes a protein of 233 amino acids. The mature protein begins at residue 77, leaving a long leader sequence of 76 amino acids. TNF-alpha has been mapped to human chromosome 6. Synonyms: Tumor necrosis factor,Cachectin,TNF-alpha,Tumor necrosis factor ligand superfamily member 2,TNF-a,Tumor necrosis factor, membrane form,N-terminal fragment,NTF,Intracellular domain 1,ICD1,Intracellular domain 2,ICD2,C-domain 1,C-domain 2,Tumor necrosis factor, soluble form,TNF,TNFA, TNFSF2,

Target Details

	Full Gene Name: Tumor necrosis factor Cellular Localisation: Cell membrane, Single-pass type II membrane protein.
Gene ID:	7124
UniProt:	P01375
Pathways:	NF-kappaB Signaling, Apoptosis, Caspase Cascade in Apoptosis, TLR Signaling, Cellular Response to Molecule of Bacterial Origin, Regulation of Leukocyte Mediated Immunity, Positive Regulation of Immune Effector Process, Production of Molecular Mediator of Immune Response, Positive Regulation of Endopeptidase Activity, Hepatitis C, Protein targeting to Nucleus, Inflammasome

Application Details

Application Notes:	Before using Kit, spin tubes and bring down all components to bottom of tube. Duplicate well assay was recommended for both standard and sample testing.
Comment:	Sequence similarities: Belongs to the tumor necrosis factor family.
Plate:	Pre-coated
Protocol:	human TNF alpha ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. A monoclonal antibody from mouse specific for TNF alpha has been precoated onto 96-well plates. Standards (E.coli,V77-L233) and test samples are added to the wells, a biotinylated detection polyclonal antibody from goat specific for TNF alpha is added subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the human TNF alpha amount of sample captured in plate.
Assay Procedure:	Aliquot 0.1 mL per well of the 1000pg/mL, 500pg/mL, 250pg/mL, 125pg/mL, 62.5pg/mL, 31.2pg/mL, 15.6pg/mL human TNF alpha standard solutions into the precoated 96-well plate. Add 0.1 mL of the sample diluent buffer into the control well (Zero well). Add 0.1 mL of each properly diluted sample of human cell culture supernates, serum or plasma(heparin, EDTA, citrate) to each empty well. See "Sample Dilution Guideline" above for details. It is recommended that each human TNF alpha standard solution and each sample be measured in duplicate.
Assay Precision:	• Sample 1: n=16, Mean(pg/ml): 93, Standard deviation: 5.1, CV(%): 5.5

Application Details

- Sample 2: n=16, Mean(pg/ml): 327, Standard deviation: 15.4, CV(%): 4.7
- Sample 3: n=16, Mean(pg/ml): 608, Standard deviation: 31, CV(%): 5.1,
- Sample 1: n=24, Mean(pg/ml): 102, Standard deviation: 7.65, CV(%): 7.5
- Sample 2: n=24, Mean(pg/ml): 319, Standard deviation: 15.3, CV(%): 4.8
- Sample 3: n=24, Mean(pg/ml): 613, Standard deviation: 35, CV(%): 5.7

Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid multiple freeze-thaw cycles.
Storage:	-20 °C,4 °C
Storage Comment:	Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles
Expiry Date:	12 months

Publications

Product cited in:

Leonel Javeres, Raza, Judith, Anwar, Habib, Batool, Nurulain: "Mixture of Organophosphates Chronic Exposure and Pancreatic Dysregulations in Two Different Population Samples." in: **Frontiers in public health**, Vol. 8, pp. 534902, (2021) (PubMed).

Liu, Xu, Chang, Qin, Yin, Yang: "4,4'-diaponeurosporene, a C30 carotenoid, effectively activates dendritic cells via CD36 and NF-κB signaling in a ROS independent manner." in: **Oncotarget**, Vol. 7, Issue 27, pp. 40978-40991, (2018) (PubMed).

Gao, Huang, Zhao, Hu, Li, Guo, Zhao, Lu: "LL202 protects against dextran sulfate sodium-induced experimental colitis in mice by inhibiting MAPK/AP-1 signaling." in: **Oncotarget**, Vol. 7, Issue 39, pp. 63981-63994, (2018) (PubMed).

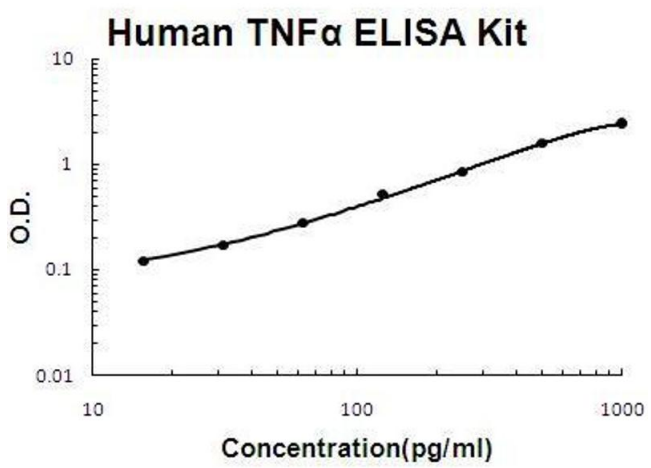
Salamanna, Borsari, Brogini, Giavaresi, Parrilli, Cepollaro, Cadossi, Martini, Mazzotti, Fini: "An in vitro 3D bone metastasis model by using a human bone tissue culture and human sex-related cancer cells." in: **Oncotarget**, Vol. 7, Issue 47, pp. 76966-76983, (2018) (PubMed).

Ma, Sun, Zhang, Zhang, Yao: "Proinflammatory effects of S100A8/A9 via TLR4 and RAGE signaling pathways in BV-2 microglial cells." in: **International journal of molecular medicine**, Vol. 40, Issue 1, pp. 31-38, (2018) (PubMed).

Publications

There are more publications referencing this product on: Product page

Images



ELISA

Image 1. Human TNF alpha PicoKine ELISA Kit standard curve

Validation report #102066 for ELISA (ELISA)

Successfully validated (ELISA (ELISA))

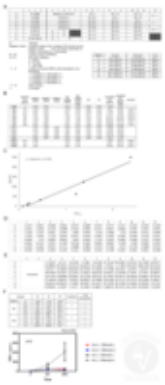
by Dept. of Radiation Oncology, Dana-Farber Cancer Institute, Harvard Medical School

Report Number: 102066

Date: Nov 16 2017

Target:	TNF
Lot Number:	23913851011
Method validated:	ELISA (ELISA)
Positive Control:	Four cell culture supernates of healthy human Peripheral Blood Mononuclear Cells (hPBMCs) stimulated by T-cell activator (anti CD3/CD28 Antibody). Healthy hPBMCs stimulated by T-cell activator are well known to release inflammatory cytokine such as TNFα and TNFγ. TNFα had been verified using a different ELISA before.
Negative Control:	Complete medium using RPMI and human serum (10%)
Spike Control:	50µl sample and 50µl TNFα standard at different concentrations diluted with 100µl sample dilution buffer (see figure)
Notes:	Passed. The human TNF ELISA kit ABIN411361 specifically recognizes TNFα in human PBMCs upon stimulation.
Standard Curve:	1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.25pg/ml, 15.625pg/ml, and 0pg/ml serial 1:2 dilution of Human TNFα standard solution in sample diluent buffer
Protocol:	• Harvest hPBMCs at different time points (before irradiation, 4h and 24h after irradiation) by centrifugation. Collect culture media and store them immediately at -20°C. • Thaw samples on ice followed by centrifugation to remove any residual particulates. • Reconstitute the human TNFα standard. Shake gently to avoid foaming. • Prepare the biotinylated anti-Human TNFα antibody working solution 30min prior to the experiment according to the kit manual. • Prepare the Avidin-Biotin-Peroxidase Complex (ABC) working solution 30min prior to the experiment according to the kit manual and keep it at 37°C until use. • Keep the TMB color developing agent and TMB stop solution at 37°C for 30min until use. • Prepare the Washing Buffer (0.01M PBS) according to the kit manual. • Prepare the standards to make 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.25pg/ml, 15.625pg/ml Human TNFα standard solutions: pipette 100µl of each of the standards, controls, and unknown samples into the assigned wells into the precoated 96-well plate. Add 100µl of the sample diluent buffer into the control well (Zero background well). Add 100µl of each sample to each empty well as indicated in figure panel A.

	• Seal the plate with a new adhesive cover provided and incubate at 37°C for 90min. • Remove the cover, discard the plate content, and blot the plate onto paper towels. The wells must not dry at any time. • Add 100µl of biotinylated anti-Human TNFα antibody working solution into each well. • Seal the plate with a new adhesive cover provided and incubate at 37°C for 60min. • Remove the cover, discard the plate content, and wash the plate 3x for 1min with 0.01M PBS. Discard the washing buffer and blot the plate onto paper towels. • Add 100µl of prepared ABC working solution into each well. • Seal the plate with a new adhesive cover provided and incubate at 37°C for 30min. • Wash the plate 5x for 1-2min with 0.01M TBS or 0.01M PBS. Discard the washing buffer and blot the plate onto paper towels. • Add 90µl of prepared TMB color developing agent into each well. • Seal the plate with a new adhesive cover and incubate at 37°C in dark for 20min. • Add 100µl of prepared TMB stop solution into each well. The color changes into yellow immediately. • Incubate for 10min after adding the stop solution. • Read the OD absorbance at 450nm in a microplate reader within 30min after adding the stop solution.
Experimental Notes:	• Samples were not diluted since a low target protein concentration was expected. • The standards and some samples were prepared in duplicates. The negative (n=1) and positive controls (n=4) were prepared in triplicates. The six different spike controls were measured without replicates (Figure panel A for details). • Increased TNFα release was detected in cell culture supernates stimulated by T cell activator without irradiation except for Sample #3. Low concentration of TNFα in all non-stimulated samples with or without irradiation were observed. We think that sample #3 was possibly a low responder for T cell stimulation (figure panel F). • Furthermore, in sample #1, TNFα level in cell culture supernate of irradiated cells (C-1) was slightly upregulated at 24 hours after irradiation compared with non-irradiated (C-3) (graph in figure panel F). Given this, although high-dose irradiation was probably enough to suppress TNFα release from the cells stimulated with T cell activator in some cells (samples #2 and #3), TNFα could be released by irradiation in some cells (sample #1).



Validation image no. 1 for Tumor Necrosis Factor alpha (TNF alpha) ELISA Kit (ABIN411361)

TNFα measurements in human PBMCs using ABIN411361. Plate setup and nature of the samples (A). OD_{450} measurements and calculated concentrations of the utilized standards and controls (B). Standard curve generated using kit standards (C). OD_{450} measurements (D), calculated concentrations (E), and summary of sample measurements (F).